Trivalent Inactivated Influenza Vaccine in African Adults Infected With Human Immunodeficient Virus: Double Blind, Randomized Clinical Trial of Efficacy, Immunogenicity, and Safety

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(See articles by Bickel et al. on pages 122–127; Crum-Cianflone et al. on pages 138–146; and editorial commentary by Dolin on pages 147–149.)

Background. Data on the efficacy of trivalent, inactivated influenza vaccine (TIV) in HIV-infected adults, particularly in Africa, are limited. This study evaluated the safety, immunogenicity, and efficacy of TIV in HIV-infected adults.

Methods. In Johannesburg, South Africa, we undertook a randomized, double-blind, placebo-controlled trial involving 506 HIV-infected adults. Subjects included 157 individuals who were antiretroviral treatment (ART) naive and 349 on stable-ART. Participants were randomly assigned to receive TIV or normal saline intramuscularly. Oropharyngeal swabs were obtained at illness visits during the influenza season and tested by shell vial culture and RT PCR assay for influenza virus. Immune response was evaluated by hemagglutinin antibody inhibition assay (HAI) in a nested cohort. The primary study outcome involved vaccine efficacy against influenza confirmed illness. This trial is registered with ClinicalTrials.gov, number NCT00757900.

Results. The efficacy of TIV against confirmed influenza illness was 75.5% (95% CI: 9.2%–95.6%); with a risk difference of 0.18 per 100 person-weeks in TIV recipients. Among TIV recipients, seroconversion, measured by HAI titers, was evident in 52.6% for H1N1, 60.8% for H3N2, and 53.6% for influenza B virus. This compared with 2.2%, 2.2%, and 4.4% of placebo recipients ($P < .0001$). The frequency of local and systemic adverse events post-immunization was similar between study groups.

Conclusions. TIV immunization is safe and efficacious in African HIV-infected adults without underlying co-morbidities. Further evaluation of effectiveness is warranted in severely immunocompromized HIV-infected adults and those with co-morbidities such as tuberculosis.

The recommendation for annual TIV immunization of HIV-infected adults since 1988 (1) is mainly based on case reports of increased illness duration and risk of influenza-associated complications in HIV-infected individuals (2, 3). TIV is generally less immunogenic in HIV-infected adults. And immunogenicity correlates directly with CD4+ cell count and inversely with HIV viral load. (4–7).

Recent meta-analysis, of 4 published studies on TIV effectiveness in HIV-infected adults, indicated a 66% [95% confidence interval (CI) 36%–82%] pooled relative risk reduction for TIV against influenza illness (8). The author's however concluded that there were

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substantial methodological shortcomings in the analyzed studies with limited evidence supporting the efficacy of TIV in HIV-infectedadults. The need for further prospective studies to guide TIV immunization policy in HIV-infectedadults, particularly in Africa where no such studies exist, was emphasized (8, 9).

We investigated in a double blind randomized trial the efficacy of TIV against influenza virus confirmed illness in adults. Additionally, our study evaluated the safety and immunogenicity of TIV.

MATERIALS AND METHODS

Study Design and Participants

HIV-infected adults, aged 18–55 years, who attended the Themba Lethu Clinic in Johannesburg, South Africa, were approached for study participation. Participants received routine HIV-related treatment according to national guidelines (10). First-line antiretroviral treatment (ART) consisted of stavudine or zidovudine, with lamivudine and either nevirapine or efavirenz. Criteria for initiating ART included WHO stage 3 or 4 HIV disease, or a CD4+ cell count less than 200 cells per microlitre.

Study-eligibility criteria included: i. first-line ART for at least 3 months, or CD4+ cell count >100 cells per microlitre within the previous 3 months if ART naïve; ii. ability to maintain weekly telephonic contact; and iii. ability to attend the clinic for illness visits. Study exclusion criteria included: i. contraindication to influenza vaccine; ii. current anti-tuberculosis treatment or history of chronic lung disease in the past 6 months; iii. contraindication to intramuscular injections; iv. existing grade 3 or grade 4 laboratory or clinical toxicity per DAIDS toxicity tables; v. previous history of receiving influenza virus or pneumococcal vaccines; and vi. systemic steroid treatment for >21 days in the past month.

Subject Enrolment and Randomization

Subjects were enrolled from 11 April 2008 to 13 June 2008. The study statistician generated a randomization list, stratified by subjects' ART status, using a computerized random-number generator (SAS 9·1, Institute Inc., Cary, NC, USA) to select random permuted group blocks of 10 with 5 in each intervention group. Participants had equal probability of assignment to groups. Enrolled subjects were allocated to the next available sequential study-number on study-entry, which was handed to the pharmacist. The pharmacist dispensed TIV or placebo according to the computer-generated randomization log provided by the statistician. Participants and all study personnel, excluding the statistician and pharmacist, were blinded to treatment assignment during the study.

Study Vaccine

The influenza vaccine recommended by WHO for the southern hemisphere in 2008 was composed of 0.5 mL suspension of split virus mixture of 15 micrograms each of A/Solomon Islands/3/2006 (H1N1)-like strain (A/Solomon Islands/3/2006 (IVR-145)), A/Brisbane/10/2007 (H3N2)-like strain (A/Brisbane/10/2007 (IVR-147)) and B/Florida/4/2006-like strain (B/Brisbane/3/2007) (11). Three lots of single-dose vials of TIV (MUTAGRIP; sanofi-aventis; Lyon, France), that is, D5091-1, DS066-1 and D002811 with expiration date of December 2008 were commercially procured.

The study pharmacist drew up 0.5 mL MUTAGRIP for the TIV group or 0.5 mL of sterile 0.9% normal-saline solution for the placebo group into a 1 mL syringe. The 2 preparations were macroscopically indistinguishable. Study-drug was administered intramuscularly by the study doctor or study nurse following randomization.

Assessment of Vaccine Efficacy

Surveillance for influenza illness started from study enrollment and continued until 30 September 2008. The onset and end of the influenza epidemic was determined by annual routine surveillance undertaken by the South Africa National Institute for Communicable Diseases (NICD) (12) The 2008 influenza epidemic in South Africa started in mid-May, peaked in mid-July, and ended at the end of August (12).

A clinical diagnosis of influenza-like illness was based on the presence of at least 3 of 4 symptom-groups of less than 7 days symptom duration. The symptom groups were: i. cough; ii. history of fever, chills, or rigor; iii. sore throat, pharyngitis, or laryngitis; and iv. myalgia or headache. Participants were sent weekly SMS reminders or telephoned to inquire and remind them about influenza-like symptoms. Subjects were requested to attend the clinic if at any stage they had features from at least 2 symptom-groups, as well as for any other inter-current illness during the study. Subjects were also able to telephone study staff to inform them of any illness or hospitalizations. Illness visits were scheduled at the clinic within 48 hours of the site being informed of illness. All subjects seen at the clinic for acute respiratory illness and subjects hospitalized for acute cardio-pulmonary illness received a clinical examination. An oropharyngeal swab was obtained from these subjects for influenza virus testing.

A flock-tipped, plastic-shaft swab (Tool and Carbide plastics (Pty) Ltd, South Africa) was used for oropharyngeal sampling. This swab was immediately immersed into viral transport medium and transported on wet ice to the NICD for testing within 12 hours. Samples were tested for influenza A and influenza B virus by shell vial culture and a qualitative one-step, real time RT PCR assay (CDC real time RT-PCR protocol for detection and characterization of influenza, CDC ref# I - 007 - 05). Influenza A virus isolates were subtyped for H1N1 and H3N2. Influenza virus identification either by shell vial culture or through real-time RT-PCR identification was considered influenza-confirmed illness.

Assessment of Vaccine Safety and Immunogenicity

Ninety-four ART-naïve and 95 adults on ART were co-enrolled into a nested immunogenicity study at the start of study-

enrollment until the targeted number had been recruited. Blood samples were obtained by venipuncture before study-vaccine administration and 1 month after vaccination. Pre-immunization CD4+ cell counts were available within 3 months of randomization. These were re-measured 1-month post-vaccination. Pre-immunization HIV-1 viral loads were available only in adults on ART, according to the standard of practice in the country. HIV-1 viral loads in adults on ART were measured by PCR (Roche Amplicor, version 1.5, Germany), with lower-detection limit of 50 copies per milliliter, 1 month post-vaccination.

Blood obtained for immunogenicity testing were centrifuged and serum was archived at -20^{o}C to -70^{o}C. Serum samples were shipped on dry ice to the CNR Virus Influenza région sud, Hospices Civils de Lyon (France), for hemagglutinin antibody inhibition assay (HAI). HAI titers of $\geq$ 1:40 if baseline titers were $<$1:10; or a $\geq$4 fold increase in those with baseline titers $\geq$1:10 were considered as evidence of seroconversion (13).

Subjects included in the immunogenicity study were provided with a diary card to record systemic and local adverse events for 4 days post-vaccination. All severe adverse events occurring at any stage during the course of the study were recorded.

Study Statistics

Based on estimates derived from the meta-analysis,(9) we predicted that 40% of placebo recipients would develop influenza-confirmed illness. Based on 0.8 power to detect a 30% reduction in influenza illness (P = .05, 2-sided), 312 individuals were required for each study group. The sample size for the nested immunogenicity study was adequate to detect seroconversion rates of at least 30% in TIV vaccinees and not more than 5% in the placebo group. The immunogenicity cohorts were separately powered based on the antiretroviral therapy status of participants at study-entry.

The primary endpoint for TIV efficacy was reduction of influenza-confirmed illness. Secondary outcome measures included clinical influenza-like illness and any acute respiratory illness. The intent-to-treat analysis of vaccine efficacy was calculated using the formula of incidence rates as $1–I_L/I_P$, (I_L = the case incidence rate in vaccinated adults with influenza sub-unit vaccine; I_P = the case incidence rate in placebo recipients). 95% CIs were constructed, and differences between the intervention arms tested at $\alpha = 0{\cdot}05$ significance level. Only the first episode of illness occurring at any time-point after receipt of study vaccine was included in each analysis.

Immunogenicity analysis within each treatment group, stratified by ART status, included calculation of geometric means of the titers (GMTs) at each sampling time-point. Treatment groups were compared to the fold-rise by a 2-sided, 2-sample t test and the corresponding 95% CIs for the ratio, all using logarithmic transformation. The proportion of subjects in the treatment groups who seroconverted were compared by $\chi 2$ tests. Differences in proportion of subjects in the treatment groups experiencing adverse events were compared with Kruskal–Wallis test. All statistical analysis was done using SAS software V9·1 (SAS Institute Inc., Cary, NC, USA).

Ethical Considerations

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand and conducted in accordance with Good Clinical Practice guidelines. Signed, written informed consent was obtained from all study participants.

The clinical trials registration number for the study is ClinicalTrial.gov number: NCT 00757900.

RESULTS

Table 1 details the demographic profile of the 506 enrolled subjects. Adults on ART had been on treatment for a median period of 25.3 months. A detailed description of the overall study population and individuals included in the nested immunogenicity study (N = 189) is shown in Figure 1. The median CD4+ counts was higher in adults on stable-ART compared with ART-naïve adults (391 vs. 277 cells per microlitre, $P < .0001$).

Trivalent Influenza Vaccine Efficacy.

The overall efficacy of TIV in preventing confirmed influenza illness was 75.5% (95% CI: 9.2% to 95.6%), with a risk reduction of 0.18 per 100 person-weeks in TIV recipients (Table 2). H1N1 virus composed 11 (91.6%) of 12 influenza cases in placebo recipients, and all 3 influenza episodes in TIV recipients. The H1N1-strain specific reduction of confirmed infection was 73.3% (95% CI: −1.2% to 95.2%) in the TIV group. There was 1 episode of influenza B virus in an ART-naïve placebo recipient.

There were no significant differences observed in clinical influenza-like illness (P = .867) or physician-attended acute respiratory infection illness (P = .402; Table 2) between TIV and placebo recipients. Three deaths occurred in the study. These included a placebo recipient who died of pneumonia within a week of study-enrollment, in whom testing for influenza was not undertaken. The cause of death in the other 2 subjects included one ART-naive placebo recipient who was diagnosed with Kaposi's sarcoma 1 month prior to death; and an ART-naïve TIV recipient who died outside of a health care facility with death being attributed to ''natural cause'' in the death registry. The latter 2 subjects died in September 2008, following the end of the 2008 influenza epidemic.

Trivalent Influenza Vaccine Immunogenicity Sub-study.

The baseline characteristics of adults included in the immunogenicity sub-study is shown in Table 3. The median CD4+ cell

Table 1. Baseline Demographic and Clinical Information for the Study Cohort Included in the Intent-To-Treat Analysis

Characteristic	Antiretroviral treatment (ART) naïve		Stable anti-retroviral treatment		Overall study population	
	TIV[a] *N* = 80	Placebo *N* = 77	TIV *N* = 175	Placebo *N* = 174	TIV *N* = 255	Placebo *N* = 251
Total follow-up time on study; person-weeks	1576.6	1528.7	3427.3	3377.4	5003.9	4906.1
Mean age (Standard deviation; SD)	34·4 (7·2)	34·6 (7·6)	37·1 (7·1)	37·2 (7·1)	36·3 (7·2)	36·4 (7·2)
Female : Male	5:1	6:1	5:1	6:1	5:1	6:1
Body mass index (SD)	24·3 (3·4)	26·2 (5·2)	25·4 (6·1)	25·3 (5·3)	25·3 (5·9)	25·4 (5·3)
Pregnant at randomization (%)	1 (1)	0	8 (5%)	4 (2%)	9 (4%)	4 (2%)
Smoker	4 (5.0%)	3 (3.8%)	14 (5.1%)	17 (9.8%)	18 (7.1%)	20 (8.0%)
Mean Hb g/dL (SD)	12·9 (2·4)	12·1 (4·6)	13·3 (1·7)	13·2 (1·7)	13·2 (7·2)	13·1 (7·9)
Median CD4 *(number observations)* within 3 months of randomization [Interquartile range; IQR]	273 (*72)* [202–353]	290 (*68)* [177–384]	384 (*170)* [280–498]	384 *(169)* [290–545]	340 (*242)* [243–473]	356 (*237)* [245–512]
Median pre-vaccination HIV viral load *(number observations)* [IQR]	Unavailable		49 (*167)* [25–49]	49 (*169)* [45–49]	Not calculated	
Proportion ART treated subjects with suppressed HIV viral load	Not applicable		157/167 (94.0%)	162/167 (97.0%)		
ART regimen at randomization: d4T-3TC-EFV d4T-3TC-NVPAZT-3TC-EFV Other	Not applicable		117 (67%) 17 (10%) 4 (2%) 36 (21%)	112 (74%) 21 (11%) 1 (1%) 28 (14%)		
Median months ART initiated before randomization	Not applicable		24 (16–38)	27 (15–41)		

[a] TIV =trivalent influenza vaccine.

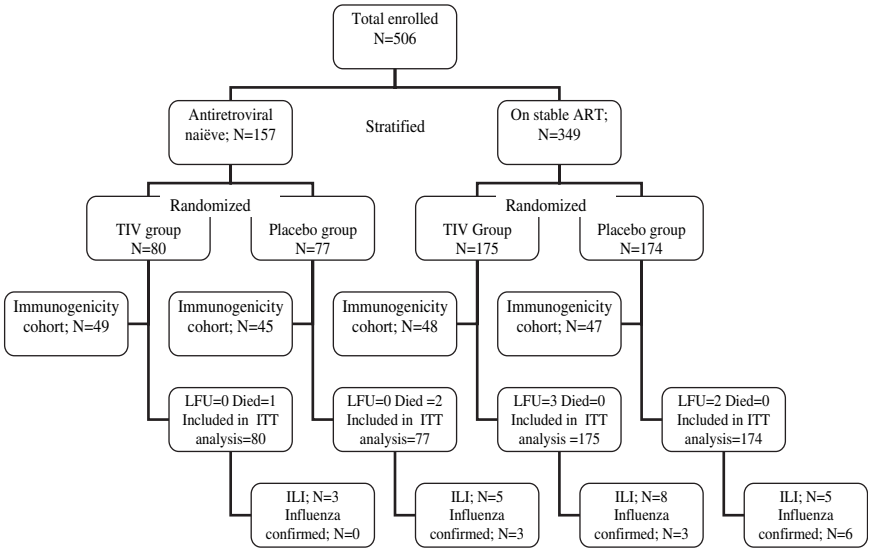

Figure 1. Consort flow diagram of study participants. Legend: ART= antiretroviral treatment. TIV= trivalent influenza vaccine. LFU= loss to follow-up. ITT =intent to treat analysis. ILI=influenza like illness.

counts were higher in adults on stable-ART compared with ART-naïve subjects (398 vs. 287 cells per microlitre, $P = .0001$; table 3). Seroconversion rates were 52.6% for H1N1, 60.8% for H3N2 and 53.6% for influenza B virus in TIV recipients; compared with 2.2%, 2.2% and 4.4% respectively in placebo recipients (Table 4; $P < .0001$). Seroconversion was, however, evident in a lower proportion of ART-naïve compared with ART-stable adults receiving TIV (table 4).

Safety of Trivalent Influenza Vaccination

The frequency of at least one solicited adverse event in the 4-day period following vaccination did not differ from TIV (14%) compared with placebo recipients (10%; Table 5). The most common reported adverse event was pain at the site of injection, which occurred in 8% of TIV recipients.

No significant changes were noted in the median CD4+ cell count 1 month post-vaccination in TIV or placebo recipients among adults on stable-ART ($P = .719$) or ART-naïve subjects ($P = .447$; Table 3). Similarly, receipt of TIV did not influence the proportion of subjects on stable-ART, who remained virologically suppressed post-immunization (table 3).

DISCUSSION

To our knowledge, this is the first randomized, controlled trial to evaluate the efficacy of TIV in African HIV-infected adults. The 75% reduction in influenza-confirmed illness in TIV-vaccinated adults in our study agrees with the 66% risk reduction of influenza illness estimated in the meta-analysis on effectiveness on TIV in HIV infected adults (8). The meta-

Table 2. Vaccine Efficacy of Trivalent Inactivated Influenza Vaccine (TIV) in Preventing Influenza Illness in HIV-Infected Adults on Stable Antiretroviral Treatment (ART) or on Stable-ART

Outcome	Antiretroviral treatment (ART) naïve		Stable-ART		Overall				
	TIV $N = 80$	Placebo $N = 77$	TIV $N = 175$	Placebo $N = 174$	TIV $N = 255$	Placebo $N = 251$	Rate reduction[b]	P value	Vaccine efficacy (95% Confidence Intervals)
Influenza Virus A or B	0 (0)[a]	6 (0·39)	3 (0·09)	6 (0·18)	3 (0·06)	12 (0·24)	0·18	0·019	75.5% (9.2–95.6)
Influenza-like illness	3 (0·19)	5 (0·33)	8 (0·23)	5 (0·15)	11 (0·22)	10 (0·20)	–0·02	0·867	–7.3% (–140.5 to 64.7)
Acute respiratory illness	14 (0·89)	15 (0.98)	26 (0·76)	32 (0.95)	40 (0·80)	47 (0.96)	0·16	0·402	16.6% (–30.0 to 46·7)
Hospitalized	0	23	34	15	3	3	Not calculated		
Died	16	23	0	0	1	2			

[a] Figures in parentheses are incidence per 100 person-weeks unless otherwise indicated.
[b] Rate reduction per 100 person-weeks. 3. Subjects hospitalized for pneumonia and another with unknown diagnosis, both of whom died. 4. One subject each hospitalized for dilated cardiomyopathy, pneumonia and another in whom no diagnosis was established, all of whom were discharged from the hospital. 5. Subjects hospitalized for gastroenteritis and discharged well. 6. 1 Subject died. Cause of death not ascertained.

Table 3. Demographic and Clinical Information For the Intensive Safety and Immunogenicity Cohort of HIV-infected Adults Receiving Trivalent Inactivated Influenza Vaccine (TIV) or Placebo

Characteristic	Antiretroviral treatment naïve group		Stable antiretroviral treatment group		Overall	
	TIV *N* = 49	Placebo *N* = 45	TIV *N* = 48	Placebo *N* = 47	TIV *N* = 97	Placebo *N* = 92
Mean age; years (Standard deviation; SD)	35·0 (7·4)	34·8 (8·2)	37·3 (7·3)	37·6 (7·0)	36·2 (7·0)	36·2 (7·0)
Female: Male	4:1	5:1	3:1	10:1	4:1	7:1
Body Mass Index (SD)	24·1 (3·2)	23·8 (6·1)	28·4 (6·9)	26·9 (5·0)	26·6 (6·4)	25·8 (1·9)
Pregnant at randomization (%)	1 (2%)	0 (0%)	3 (6%)	3 (6%)	4 (4%)	3 (3%)
Smoker (%)	2 (4%)	3 (7%)	6 (13%)	5 (11%)	8 (8%)	8 (9%)
Median CD4+ cell counts per μl within three months of randomization [Interquartile range; IQR]	278 [178–325]	288 [174–358]	419 [294–500]	384 [285–584]	321 [223–456]	326 [232–501]
Median pre-vaccination HIV viral load within three months of randomization *(number of observations)* [IQR]	Unavailable		49 (*47)* [49–49]	49 (*46)* [49–49]	Not calculated	
Proportion suppressed viral load pre vaccination n (%)	Not applicable		43/47 (91%)	41/46 (89%)	Not applicable	
Antiretroviral treatment regimen at randomizationd 4T-3TC-EFV d4T-3TC-NVPAZT-3TC-EFV Other	Not applicable		33 (69%) 6 (13%) 2 (4%) 7 (14%)	31 (66%) 7 (15%) 0 (0%) 9 (19%)	Not applicable	
Median period, months, time from ART initiation to randomization (IQR)	Not applicable		24 (15–36)	24 (12–41)	Not applicable	
Median post-vaccination viral load –copies per milliliter *(number of observations)* [IQR]	3 300 (*49)* [570–13000]	3 400 (*43)* [1400–17000]	49 (*46)* [49–49]	49 (*47)* [49–49]	49 (*95)* [49–3800]	150 (*90)* [49–4700]
Suppressed viral load post vaccination (%)	Not applicable		44/47 (94)	44/46 (96)	Not applicable	
Median post-vaccination CD4+ count *(number of observations)* [IQR]	246 (*49)* [195–375]	280 (*43)* [185–368]	412 (*46)* [305–513]	387 (*47)* [275–534]	325 (*95)* [221–459]	319 (*90)* [235–439]
Median difference in CD4+ count 28 days post-vaccination *(number of observations)* [IQR]	1 (*44)* [−45 to 52]	−13 (*40)* [−41 to 18]	4 (*46)* [−78 to 59]	1 (*44)* [−45 to 52]	−9 (*46)* [−68 to 51]	−12 (*86)* [−48 to 23]

Table 4. Seroconversion Rates Measured by Hemagglutinin Inhibition Assay (HAI) to Influenza Virus Strains in Antiretroviral (ART) Naive and ART-Stable HIV-Infected Adults Randomized to Trivalent Inactivated Influenza (TIV) Vaccine or Placebo

	ART naïve			Stable-ART			Overall		
Immunogenicity measure	TIV $N = 49$	Placebo $N = 44$	P value	TIV $N = 48$	Placebo $N = 47$	P value	TIV $N = 97$	Placebo $N = 91$	P value
H1N1 HAI assay									
Seroconverison (%)[a]	17 (34.7)[d]	0 (0)	<.0001	34 (70.8)[d]	2 (4.3)	<.0001	51 (52.6)	2 (2.2)	<.0001
Pre-vaccine GMT (95% CI)	6.3 (5.4,7.2)	8.3 (6.3,10.8)	.069	9.4 (7.4,12.0)	7.6 (6.2,9.2)	.151	7.7 (6.7,8.8)	7.9 (6.7,9.3)	.794
Post-vaccine GMT (95% CI)	25.8 (17.5,38.9)	8.1 (6.3,10.5)	<.0001	64.4 (41.3,100.6)	8.5 (6.8,10.7)	<.0001	40.6 (29.955.1)	8.3 (7.1,9.8)	<.0001
Mean GMT fold increase (95%CI)	4.1 (2.8,8.1)	1.0 (0.9,1.1)	<.0001	6.8 (4.2,11.0)	1.1 (0.9,1.4)	<.0001	5.3 (3.9,10.1)	1.1 (0.9,1.2)	<.0001
H3N2 HAI assay									
Seroconverison (%)	25 (51.0) [d]	0 (0)	<.0001	34 (70.8) [d]	2 (4.3)	<.0001	59 (60.8)	2 (2.2)	<.0001
Pre-vaccine HAI titre GMT (95% CI)	8.9 (6.9,11.5)	7.5 (5.8,9.8)	.351	14.3 (10.4,19.8)	15.6 (11.0,21.9)	.729	11.3 (9.2-,13.9)	11.0 (8.7,13.7)	.846
Post-vaccine HAI titre GMT (95% CI)	48.8 (30.0,79.1)	7.7 (5.9,10.0)	<.0001	146.7 (80.2,268.4)	16.8 (11.9,23.6)	<.0001	84.1 (56.6,124.9)	11.5 (9.1,14)	<.0001
Mean GMT fold increase (95%CI)	5.5 (3.7,8.1)	1.0 (0.98,1.0)	<.0001	10.2 (6.4,16.2)	1.1 (0.9-,1.4)	<.0001	7.4 (5.5,10.1)	1.0 (0.96,1.1)	<.0001
Influenza B HAI assay									
Seroconversion	22 (44.9) [d]	1 (2.3)	<.0001	30 (62.5)[d]	3 (6.4)	<.0001	52 (53.6)	4 (4.4)	<.0001
Pre-vaccine HA titre GMT (95%CI)	8.8 (6.7,11.5)	8.4 (6.3,11.2)	.8146	10.9 (8.5,14.0)	9.2 (6.9,12.2)	.3576	9.8 (8.2,11.7)	8.8 (7.2,10.7)	.4269
Post-vaccine HA titre GMT (95% CI)	39.4 (25.3,61.4)	9.2 (6.9,12.4)	<.0001	69.2 (46.3,103.6)	10.9 (7.9,15.0)	<.0001	52.1 (38.6,70.3)	10.1 (8.1,12.5)	<.0001
Mean GMT fold increase (95%CI)	4.5 (3.1,6.6)	1.1 (1.0,1.2)	<.0001	6.3 (4.3,9.5)	1.2 (1.0,1.4)	<.0001	5.3 (4.0,7.0)	1.1 (1.0,1.3)	<.0001

[a] Seroconversion defined as: HAI titers of ⩾ 1:40 if baseline titers were <1:10; or a ⩾4 fold increase in those with baseline titers ⩾1:10.(12).

[b] GMT=Geometric mean titers of hemagglutinin-inhibition antibody. 95% Confidence intervals (95%CI) shown in parenthesis.

[c] Measure of fold increase in GMT comparing pre- to post- vaccine HAI titres.

[d] P-value comparing seroconversion rates between ART-naiëve vs. ART-stable individuals who received TIV: H1N1: $P = .0015$), H3N2: $P = .046$; influenza B virus: $P = .082$.

Table 5. Solicited Adverse Events Following Trivalent Inactivated Influenza Vaccine (TIV) or Placebo in HIV-Infected Adults Naïve to Antiretroviral Treatment (ART-Naïve) or on Stable-ART

	ART-naïve			Stable-ART			Overall		
Safety measures	TIV $N = 49$	Placebo $N = 44$	P value	TIV $N = 48$	Placebo $N = 47$	P value	TIV $N = 97$	Placebo $N = 91$	P value
Any adverse events	7 (14.2)[a]	4 (9.1)	.438	7 (14.5)	5 (10.6)	.562	14 (14.4)	9 (9.9)	.342
Pain	4 (8.2)	2 (4.5)	.481	4 (8.3)	2 (4.3)	.416	8 (8.3)	4 (4.4)	.282
Redness	2 (4.1)	0 (0)	.178	0 (0)	1 (2.1)	.312	2 (2.1)	1 (1.1)	.599
Swelling	1 (2.0)	0 (0)	.343	1 (2.1)	0 (0)	.322	2 (2.1)	0 (0)	.170
Lump formation	1 (2.0)	1 (2.3)	1.000	0 (0)	1 (2.1)	.312	1 (1.0)	2 (2.2)	.611
Bruising	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
Itching	2 (4.1)	0 (0)	.178	1 (2.1)	1 (2.1)	1.000	3 (3.1)	1 (1.1)	.345
Rigors	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
Fatigue	1 (2.0)	3 (6.8)	.260	0 (0)	2 (4.3)	.151	1 (1.0)	5 (5.5)	.083
Headache	1 (2.0)	0 (0)	0.343	2 (4.2)	3 (6.4)	.630	3 (3.1)	3 (3.3)	1.000
Fits	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
Myalgia	3 (6.1)	1 (2.3)	.364	0 (0)	1 (2.1)	.312	3 (3.1)	2 (2.2)	.703
Arthralgia	3 (6.1)	2 (4.5)	.738	0 (0)	2 (4.3)	.151	3 (3.1)	4 (4.4)	.638
Fever	0 (0)	0 (0)	1.000	0 (0)	1 (2.1)	.312	0 (0)	1 (1.1)	.301

[a] Values in parentheses are percentages.

analysis included a single randomized-controlled trial undertaken among 102 US military recruits (8), and 3 other uncontrolled studies graded as having major methodological limitations (8, 9, 14).

Our study of the vaccine efficacy and influenza attack rates observed when directly compared to other published research are further confounded by difference outcomes between studies. Whilst our study focused primarily on a specific outcome of confirmed influenza illness, other studies have variably measured TIV effectiveness against less specific composite outcomes such as any respiratory illness, clinical influenza like illness, serological outcomes and only infrequently was influenza virus identification confirmed (14–16).

Although our study reports a high point-efficacy estimate, the study nevertheless has limitations. These include the low attack rate of confirmed influenza illness observed among controls (4.8%) and wide 95% confidence intervals (9.2%–95.6%) of the vaccine efficacy estimate. Fluctuations in the severity of influenza epidemics, based on the virulence of the circulating influenza strain in a particular season are inherently unpredictable and uncontrollable factors in TIV efficacy trials. An additional limitation of our study relates to the under-enrollment of the number of subjects that we had originally targeted. This was partially offset by a lower loss to follow-up (.99%) than the 20% that had been anticipated in the initial sample-size calculation. Limited resources of this investigator sponsored study prevented extending our study to involve multiple centers.

The vaccine efficacy estimate in our study may have been negatively affected because of a drift from the H1N1 influenza strain included in TIV compared with the H1N1 strain that circulated during the epidemic. Antigenic testing at the NICD indicated that the South African H1N1 influenza viruses in 2008 reacted to low titers against A/SolomonIslands/3/2006-like virus anti-sera and reacted well against A/Brisbane/59/2007-like antisera. This antigenic drift away from the vaccine H1N1 strain was confirmed by partial sequencing of the HA1 subunit of hemagglutinin (17).

Although there were 3 episodes of hospitalization in each of our study groups, including 2 deaths in the placebo group and 1 death in the TIV group, none of these episodes were confirmed to be a result of the influenza virus. Similarly, none of the influenza illnesses observed in the TIV studies undertaken in the US military or Japan required hospitalization (14, 15). A United Sates study, however, indicated that although influenza-associated hospitalizations had declined in patients with HIV infection in the post-HAART era, the rates remained comparable to rates in other high-risk groups for which annual influenza vaccination was recommended (18).

The seroconversion rates in our study were moderate and similar to other studies of asymptomatic, HIV-infected adults (52%–89%), but lower than the 94%–100% observed in HIV-uninfected adults (4). TIV-induced seroconversion rates in adults with AIDS range between 13% and 50% (4). The seroconversion rates to H1N1 (52.6%) in our study, however, under-estimated actual protection against confirmed H1N1 illness (73%). This highlights the potential of under-estimating the likely benefit of TIV immunization when relying solely on humoral immunogenicity markers, particularly as poor humoral

antibody responses have been documented in other studies of HIV-infected adults (7, 19).

The short-term safety of TIV in our study-population was supported by the absence of differences in solicited adverse-event rates, changes in CD4+ cell count, or HIV viral control (in ART-stable subjects) between TIV vaccinees compared with placebo recipients. This allays previous concerns regarding TIV vaccination being associated with transient increase in HIV-1 viral load and decline in CD4+ cell count, even in the presence of ART in virologically suppressed individuals (20–24). A limitation of our study was the fct that we did not monitor HIV viral load in HIV-infected adults not on ART.

This was a single-center study, with entry criteria limited to those without co-morbidities. And the study was conducted over a single influenza season. But our study nevertheless provides the most compelling evidence of TIV efficacy in HIV-infected adults. In light of our positive findings, we believe the effectiveness of TIV needs further evaluation in HIV-infected adults with other underlying co-morbidities and risk factors. This includes individuals with tuberculosis, chronic lung disease, and those with severe immunosupression in whom poor humoral immune responses to TIV have been observed when CD4+ cell counts are $<$100 cells per microlitre (6).

These high-risk groups were excluded from our study to minimize potential confounders that could have affected evaluating the safety and efficacy of TIV in this initial study involving African HIV-infected adults (25). Similarly, our protocol excluded individuals previously immunized with TIV or pneumococcal polysaccharide vaccine. Exclusion of adults previously vaccinated with TIV was based on the possibility that previous TIV vaccination may affect its immunogenicity and effectiveness (26), and uncertainty about the long-term effect of repeat or annual TIV immunization (27). Exclusion of individuals previously immunized with pneumococcal polysaccharide vaccine, which is not standard of care in Africa because of uncertainty of its safety and efficacy in African HIV-infected adults (28), was based on the potential interaction of influenza virus and *Streptococcus pneumoniae* in causing severe influenza-related illness (29). A study in HIV-infected children indicated that pneumococus-conjugate vaccine immunization reduced hospitalization rates of severe influenza-associated illness (30).

While TIV (and pneumococcal polysaccharide vaccine) are available in South Africa and other African countries, vaccine use is limited in these settings because of lack of safety and efficacy data. Our study has however established TIV to be safe in the immediate period after vaccination. We showed that TIV is immunogenic and efficacious in HIV-infected Africans with CD4+ cell counts $>$100 cells per microlitre and absent underlying co-morbidities. Further studies are required to address the effectiveness of TIV across more severe hospitalization outcomes, as well as the effectiveness and safety of repeat or annual immunization. The results from our study, nevertheless, indicate that TIV immunization should become standard of care in HIV-infected adults in sub-Saharan Africa.

CONTRIBUTORS

SAM, AK, LK, TGB, CC, CLC, and IS participated in the conception of the trial, study design, protocol development, and study planning and implementation. SAM, AK, and IS followed up participants and gathered data. TGB and DN managed laboratory set-up and sample processing. MV was involved in undertaking the immunogenicity analysis. SAM, MM, MV, and IS analyzed data. All authors participated in interpretation of the results. SAM, MM, TGB, DN, and IS drafted the report, and all authors contributed to critical review and report revision. All authors have seen and approved the final report version.

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